FILE NO.A332626-A 067252.0107
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Smith et al.			
Serial No.	:	TBA	Examiner	:	TBA
Filed	:	July 20, 2001	Group Art Unit:		TBA
For	:	RECOMBINANT VACCINE AGAINST BOTULINUM NEUROTOXIN			

PRELIMINARY AMENDMENT AND
SUBMISSION OF SEQUENCE LISTING

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants respectfully request consideration of the above-identified application in light of the following amendments and remarks. Applicants submit herewith a Sequence Listing in paper and computer form.

IN THE SPECIFICATION

Please **amend** the paragraph beginning at page 1, line 3 and ending at page 1, line 6 with the following rewritten paragraph:

This application is a continuation of U.S. Non-Provisional Application 09/611,419 filed July 6, 2000 which is based on U.S. Provisional Applications Nos. 60/133,866, 60/133,868, 60/133,869, 60/133,865, 60/133,873, and 60/133,867, all filed

May 12, 1999, U.S. Provisional Application No. 60/146,192, filed July 29, 1999, and U.S. Patent Application No. 08/123,975, filed September 21, 1993, all of which are incorporated herein by reference in their entirety.

Please **amend** the paragraph beginning at page 21, line 20 and ending at page 21, line 29 with the following rewritten paragraph:

Analysis of CD spectra of both soluble and resolubilized product revealed the presence of significant β-sheet which is in agreement with that predicted for rBoNTF(H_C) using an artificial neural network (Lebeda, F.J., et al., (1997), "Predicting Differential Antigen-Antibody Contact Regions Based on Solvent Accessibility," *J. Protein Chem.*, **16:**607-618), and that determined by crystal structure of BoNT serotype A (Lacy, D.B., et al., (1998), "Crystal Structure of Botulinum Neurotoxin Type A and Implications for Toxicity," *Nat. Struct. Biol.*, **5:**898-902). However, even though CD revealed that the two antigens possessed similar folds, there were subtle differences between the two spectra suggesting that the secondary structures, and hence, tertiary structures were not identical.

At page 45, following the heading "CLAIMS", please **insert** the following on a new line:

--We claim:--

IN THE CLAIMS

Please **cancel** claims 1-38.

Please **add** new claims 39-86.

39. (NEW) A nucleic acid comprising a nucleic acid sequence which encodes the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G and is capable of being expressed in an organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line.

40. (NEW) The nucleic acid of claim 39, wherein the gram negative bacteria is *Escherichia coli*.

41. (NEW) The nucleic acid of claim 39, wherein the yeast is *Pichia pastoris*.

42. (NEW) The nucleic acid of claim 39, wherein said nucleic acid comprises a nucleic acid sequence selected from the group consisting of SEQ ID No. 7, SEQ ID No. 9, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 15, and SEQ ID No. 17.

43. (NEW) A nucleic acid comprising a sequence which encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID No. 8, SEQ ID No. 10, SEQ ID No. 12, SEQ ID No. 14, SEQ ID No. 16, and SEQ ID No. 18.

44. (NEW) The nucleic acid of claim 39, wherein said nucleic acid is a synthetic nucleic acid.

45. (NEW) The nucleic acid of claim 39, wherein said nucleic acid is operably linked to expression control sequences.

46. (NEW) The nucleic acid of claim 39, wherein said expression control sequences comprise a promoter.

47. (NEW) The nucleic acid of claim 39, wherein said expression control sequences comprise an enhancer.

48. (NEW) A method of preparing a polypeptide comprising the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G, said method comprising

transfecting an organism with the nucleic acid of claim 39;

culturing the transfected organism under conditions wherein the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype is expressed, wherein the organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line.

49. (NEW) The method of claim 48, further comprising recovering insoluble protein from said transfected organism.

50. (NEW) The method of claim 48, wherein said organism is *Escherichia coli*.

51. (NEW) The method of claim 48, wherein said organism is *Pichia pastoris*.

52. (NEW) An immunogenic composition comprising the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G.

53. (NEW) A method of preparing the immunogenic composition of claim 52, said method comprising culturing a recombinant host organism transfected with an expression vector encoding, in an expressable form, the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype.

54. (NEW) A method of preparing the immunogenic composition of claim 52 comprising

culturing a recombinant organism capable of expressing the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype and

recovering an insoluble protein fraction from the recombinant organism.

55. (New) The nucleic acid of claim 39, wherein the A+T content is less than about 70% of the total base composition.

56. (New) The nucleic acid of claim 55, wherein the A+T content is less than about 60% of the total base composition.

57. (NEW) A nucleic acid comprising a nucleic acid sequence which encodes the amino-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G and is capable of being expressed in an organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line.

58. (NEW) The nucleic acid of claim 57, wherein the gram negative bacteria is *Escherichia coli*.

59. (NEW) The nucleic acid of claim 57, wherein the yeast is *Pichia pastoris*.

60. (NEW) The nucleic acid of claim 57, wherein said nucleic acid comprises a nucleic acid sequence selected from the group consisting of SEQ ID No. 21, SEQ ID No. 23, SEQ ID No. 25, SEQ ID No. 27, SEQ ID No. 29, and SEQ ID No. 31.

61. (NEW) A nucleic acid comprising a sequence which encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID No. 22, SEQ ID No. 24, SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 30, and SEQ ID No. 32.

62. (NEW) The nucleic acid of claim 57, wherein said nucleic acid is a synthetic nucleic acid.

63. (NEW) The nucleic acid of claim 57, wherein said nucleic acid is operably linked to expression control sequences.

64. (NEW) The nucleic acid of claim 57, wherein said expression control sequences comprise a promoter.

65. (NEW) The nucleic acid of claim 57, wherein said expression control sequences comprise an enhancer.

66. (New) The nucleic acid of claim 57, wherein the A+T content is less than about 70% of the total base composition.

67. (New) The nucleic acid of claim 66, wherein the A+T content is less than about 60% of the total base composition.

68. (NEW) A method of preparing a polypeptide comprising the amino-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G, said method comprising

transfecting an organism with the nucleic acid of claim 57,

culturing the transfected organism under conditions wherein the amino-terminal portion of the heavy chain of a botulinum neurotoxin serotype is expressed, wherein the organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line.

69. (NEW) The method of claim 68, further comprising recovering insoluble protein from said transfected organism.

70. (NEW) The method of claim 68, wherein said organism is *Escherichia coli*.

71. (NEW) The method of claim 68, wherein said organism is *Pichia pastoris*.

72. (NEW) An immunogenic composition comprising the amino-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G.

73. (NEW) A method of preparing the immunogenic composition of claim 72, said method comprising culturing a recombinant host organism transfected with an

expression vector encoding, in an expressable form, the amino-terminal portion of the heavy chain of a botulinum neurotoxin serotype.

74. (NEW) A method of preparing the immunogenic composition of claim 72 comprising

culturing a recombinant organism capable of expressing the amino-terminal portion of the heavy chain of a botulinum neurotoxin serotype and

recovering an insoluble protein fraction from the recombinant organism.

75. (NEW) An immunogenic composition comprising at least a portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G.

76. (NEW) The immunogenic composition of claim 75, wherein said portion of the heavy chain of a botulinum neurotoxin serotype elicits an ELISA response to the corresponding botulinum neurotoxin serotype in an animal, said ELISA response being detectable upon about 100-fold dilution of serum from said animal.

77. (NEW) The immunogenic composition of claim 75, wherein said portion of a botulinum neurotoxin serotype comprises at least one epitope of the amino-terminal protion or the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G, wherein said epitope is capable of eliciting protective immunity toward the corresponding botulinum neurotoxin serotype.

78. (NEW) The immunogenic composition of claim 77, wherein said immunogenic composition elicits an ELISA response to a botulinum neurotoxin serotype in an animal, said ELISA response being detectable upon about 100-fold dilution of serum from said animal.

79. (NEW) The immunogenic composition of claim 75, wherein said composition is endotoxin free.

80. (NEW) A nucleic acid encoding a protein comprising at least one epitope of the amino-terminal protion or the carboxy-terminal portion of the heavy chain of a botulinum neurotoxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G.

81. (NEW) The nucleic acid of claim 80, wherein said protein is a fusion protein further comprising a non-toxic polypeptide sequence.

82. (NEW) A recombinant host cell comprising the nucleic acid of claim 39, 57, or both.

83. (NEW) The recombinant host cell of claim 82, wherein said host cell expresses a protein comprising at least a portion of the heavy chain of a botulinum neurotxin serotype selected from the group consisting of serotype B, serotype C_1, serotype D, serotype E, serotype F, and serotype G.

84. (NEW) The recombinant host cell of claim 83, wherein said protein elicits an ELISA response to a botulinum neurotxin serotype in an animal, said ELISA response being detectable upon about 100-fold dilution of serum from said animal.

85. (Original) The recombinant host cell of claim 82, wherein said protein is at least 0.75% (w/w) of the total cellular protein.

86. (Original) The recombinant host cell of claim 85, wherein said protein is at least 20% (w/w) of the total cellular protein.

REMARKS

Applicants respectfully request consideration of the above-identified application in light of the following amendments and remarks.

Upon entry of this amendment, Claims 39-86 will be pending in the instant application. Applicants believe that all original claims are in condition for allowance. However, in preparation for interference procedings, Claims 1-38 have been canceled and Claims 39-86 have been added to remove references to botulinum neurotoxin serotype A from the instant application. Applicants intend to pursue subject matter pertaining to serotypes A in one or more other applications. Consequently, the instant Preliminary Amendment, should not be construed as surrendering any subject matter. Applicants do not concede surrender of any subject matter recited by the original claims pending review of any related application(s) upon its merits.

New Claims 39-86 are all supported by the specification as originally filed *inter alia* Claims 1-38 and do not constitute new matter.

Rewritten specification paragraphs appear in the preceding "IN THE SECIFICATION" section. Attached hereto is a marked-up version of the changes made by

the instant amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" and is only included for the Examiner's convenience. Should any discrepancies be discovered, the version presented in the preceding "IN THE SPECIFICATION" section shall be deemed to be correct.

Applicants submit herewith a Sequence Listing in paper and computer form. I hereby state that the content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e), are the same. I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(g), herein does not include new matter.

Applicants do not believe any fee is required for this filing. Nevertheless, the Commissioner is hereby authorized to charge any fees due with this submission not otherwise enclosed herewith to Deposit Account No. 02-4377. Two copies of this paper are enclosed.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the following sections, added text is marked with double underlining. e.g. added text, and deleted text is marked by a single strikethrough, e.g. ~~deleted text~~.

IN THE SPECIFICATION

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